植 物 分 类 与 资 源 学 报 2015, 37 (4): 439~452
Plant Diversity and Resources DOI: 10.7677/ynzwyj201514126

不同居群紫花针茅响应干旱胁迫的生理和分子差异分析*

李 雄[1,2,3], 杨时海[3,4], 杨云强[1,2], 尹 欣[1,2,3], 孙旭东[1,2], 杨永平[1,2**]
(1 中国科学院昆明植物研究所东亚植物多样性与生物地理学重点实验室, 昆明 650201; 2 中国科学院中国西南野生生物种质资源库, 昆明 650201; 3 中国科学院大学, 北京 100049; 4 中国科学院青藏高原研究所, 北京 100101)

摘要: 紫花针茅 (*Stipa purpurea*) 在青藏高原沿广阔的降水梯度分布。长期的适应过程中, 不同地域的紫花针茅可能对干旱具有不同的响应方式。本研究以两个居群的紫花针茅为材料, 对干旱胁迫 14 天和随后的复水过程中各自的生理和分子水平的变化差异进行研究。结果发现两个居群的紫花针茅在植株死亡率、叶片相对含水量、叶绿素荧光、活性氧积累、脯氨酸含量、抗氧化酶活性和抗旱相关基因表达量的变化等方面都有明显差异; 且复水后各项指标的恢复水平也不相同。分析表明来自降水较少的普兰地区的紫花针茅的后代与来自降水较多的措勤地区的相比表现出更强的耐旱性, 说明不同居群的紫花针茅对干旱的响应差异可能是遗传性。本研究有助于认识紫花针茅在环境中的适应和进化, 以及对气候变化的响应。

关键词: 紫花针茅; 青藏高原; 干旱; 适应和进化; 气候变化
中图分类号: Q 945 **文献标志码**: A **文章编号**: 2095-0845(2015)04-439-14

Comparative Physiological and Molecular Analyses of Intraspecific Differences of *Stipa purpurea* (Poaceae) Response to Drought*

LI Xiong[1,2,3], YANG Shi-hai[3,4], YANG Yun-qiang[1,2], YIN Xin[1,2,3],
SUN Xu-dong[1,2], YANG Yong-ping[1,2**]
(1 *Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Sciences*, Kunming 650201, China; 2 *Germplasm Bank of Wild Species in Southwest China, Kunming Institute of Botany, Chinese Academy of Sciences*, Kunming 650201, China; 3 *University of Chinese Academy of Sciences*, Beijing 100049, China; 4 *Institute of Tibetan Plateau Research, Chinese Academy of Sciences*, Beijing 100101, China)

Abstract: *Stipa purpurea* Griseb, the dominant species of alpine steppe, is widely distributed across the large precipitation gradient on the Tibetan Plateau. It is possible that because of local adaptation, *S. purpurea* populations from different habitats along this precipitation gradient respond differently to drought, which may affect their responses to climate change. To explore the problem, in the present study, we investigated the physiological and molecular response of *S. purpurea* seedlings from two different populations (Pulan & Cuoqin) to 14-d drought stress and subsequent recovery. The results showed that the relative water content, chlorophyll fluorescence, content of osmoticant proline and malondialdehyde (indicator of oxidative stress), accumulation of reactive oxygen species, antioxidant enzyme activities and the expression of drought-related genes all changed under drought treatment and went back to the control levels in the subsequent recovery in plants from Pulan. However, these patterns were quite different in plants from Cuoqin, in which these traits changed by inconsistent degrees and did not return to pretreatment levels after rewatering. The results demonstrated that the plants from Pulan had greater resistance to drought stress compared with those from Cuoqin, which had a larger mortality rate ultimately. Combating the differences of offspring in

* Funding: The National Natural Sciences Foundation of China (41271058) and the Major State Basic Research Development Program (2010CB951700)
** Author for correspondence; E-mail: yangyp@mail.kib.ac.cn
Received date: 2014-09-12, Accepted date: 2014-11-27
作者简介: 李雄 (1987-) 男, 博士研究生, 主要从事植物生理生态学和分子生物学研究。E-mail: lixiong@mail.kib.ac.cn

response to drought and the habitat distribution of parents, we considered that genetic basis has been obtained in response to precipitation difference among *S. purpurea* populations. The results help to understand the adaptation and evolution of *S. purpurea* to the special environment and the effect of climate change to this botanical system.

Key words: *Stipa purpurea*; Tibetan Plateau; Drought; Adaptation and evolution; Climate change

Stress constantly challenges the survival and development of plants, and plants have in turn evolved many adaptations to different environments. Among the different stresses, drought stress draws much attention because it is a major factor that decreases plant growth and productivity (Yordanov *et al.*, 2000; Aranjuelo *et al.*, 2011). Plants endure drought stress mainly by avoiding tissue dehydration, while maintaining as high tissue water potential as possible, or by tolerating low tissue water potential. In fact, plants initiate an integrative mechanism during this process, involving phenotypic plasticity, alterations in biochemical enzyme activity or proteomic dynamics, and stress-responsive changes in gene expression (Nicotra and Davidson, 2010). Under drought stress, water loss in plants can be minimized by rolling leaves (Turgut and Kadioglu, 1998; Terzi *et al.*, 2013), thick leaves (Hu *et al.*, 2012), developed trichomes (Fu *et al.*, 2013), or more rigid cell walls (Moore *et al.*, 2008) etc. In addition, the plant can increase the root investment, such as increasing the root depth (Moore *et al.*, 2008). Consistent with phenotypic plasticity, physiological and biochemical adjustments are involved in the tolerance to a low tissue water potential (Chaves *et al.*, 2003). Plants close their stomata to reduce water transpiration and photosynthesis when exposed to drought (Chaves *et al.*, 2003). The osmotic compounds, like proline, also regulate osmotic adjustment in plant adaptation to drought (Morgan, 1984). A series of scavenging mechanisms, including antioxidant enzymes, can restrict the over-accumulation of reactive oxygen species (ROS), which can damage cell membranes and other cellular components during drought stress (Scandalios, 1993). Correlated with both morphological and physiological divergence, water shortage is reported to trigger drought-responsive gene expression, which is characterized by the induction of signal transduction (Yamaguchi-Shinozaki and Shinozaki, 2004, Huang *et al.*, 2012), channel protein genes (Johanson *et al.*, 2001; Luu *et al.*, 2007; Danielson and Johanson, 2008), transcription factors (Nelson *et al.*, 2007; Qiu and Yu 2009; Tripathi *et al.* 2014), and other defense genes (Hare *et al.*, 1999; Catala *et al.*, 2007; Sun *et al.*, 2013), to subsequently maintain homeostasis (Fordyce, 2006). However, plant drought stress responses are complex biological processes that are controlled by multiple trait loci, and most studies to date have focused on model and crop plants, neglecting alpine plants which survive under harsh environmental stress and are possibly highly valuable for their potential resistance genetic resources. Clearly, many challenges remain for the comprehensive understanding of the mechanism of plant tolerance to drought stress.

Climate warming has caused substantial changes in temporal and spatial precipitation patterns in many regions of the world (Zhuang *et al.*, 2010). The Tibetan Plateau, famous as "The third pole" and "Roof of the world", is closely connected with global climate change (Carlyle *et al.*, 2014; Wang *et al.*, 2014). Although there are plentiful freshwater resources in the Tibetan Plateau, and this region serves as the headwater for many of Asia's rivers (Wang *et al.*, 2009), the distribution of the water resources is predominantly dependent on its topographic configuration and atmospheric circulation pattern. The present atmospheric circulation in Asia is dominated by a westerly and monsoon wind system, which results in an approximate rainfall gradient from east to west on the Tibetan Plateau (Klein *et al.*, 2004; Shen *et al.*, 2008), with accompanying changes in ecosystem types from alpine meadow,

steppe to desert (Ni, 2000). Meanwhile, global climate change and human activities are potential reasons for the accelerating drought trend on the Tibetan Plateau. Tree-line data indicates that extreme drought conditions have progressed into the northwestern Tibetan Plateau during the past 300 years (Gou *et al.*, 2006, 2007). Therefore, rainfall is one of the most important factors determining the structure and function of plant populations, communities and alpine ecosystems on the Tibetan Plateau, indicating that relevant studies should be very helpful for ecosystem management, conservation, and development. However, most previous studies have focused on macroecological effects (Bothe *et al.*, 2010, 2011; Zhu *et al.*, 2011; Yang *et al.*, 2012; Sun and Liu, 2013; Wu *et al.*, 2013; Yang *et al.*, 2013; Wang *et al.*, 2013; Fang *et al.*, 2014), whereas very little is known with regard to the effects of rainfall on the evolution and adaptation of the particular species or populations on the Tibetan Plateau at the physiological and molecular levels.

Stipa purpurea Griseb. is an endemic and dominant perennial grass species in the alpine steppe and meadow environments that are widely distributed in the Tibetan Plateau, Pamirs Plateau and high mountains in Central Asia (Yue *et al.*, 2011). Because of the wide distribution and strong resistance to cold, drought and high winds (Zhou *et al.*, 1987), it plays a vital role in safeguarding soil and water resources, acting as a windbreak and preventing erosion (Yue *et al.*, 2011). With global climate change, *S. purpurea* is an available candidate to study alpine plants response to drought stress and mine the resistance genetic resources. As *S. purpurea* has an extremely wide range of distributions on the Tibetan Plateau, it may be locally adapted to regional environmental variation. In the present study, we investigated the physiological and molecular divergence of plants of *S. purpurea* from two population response to drought stress in the laboratory and analyzed how the difference was correlated with their respective phenotype and acclimation to habitat environments. We supposed that the *S. purpurea* population living in the more arid regions might have relatively stronger tolerance to the same degree of drought as a result of heredity effect. The results should help us to better understand how physiological and molecular divergence contributes to drought adaptation across natural populations of *S. purpurea* on the Tibetan Plateau.

1 Materials and methods

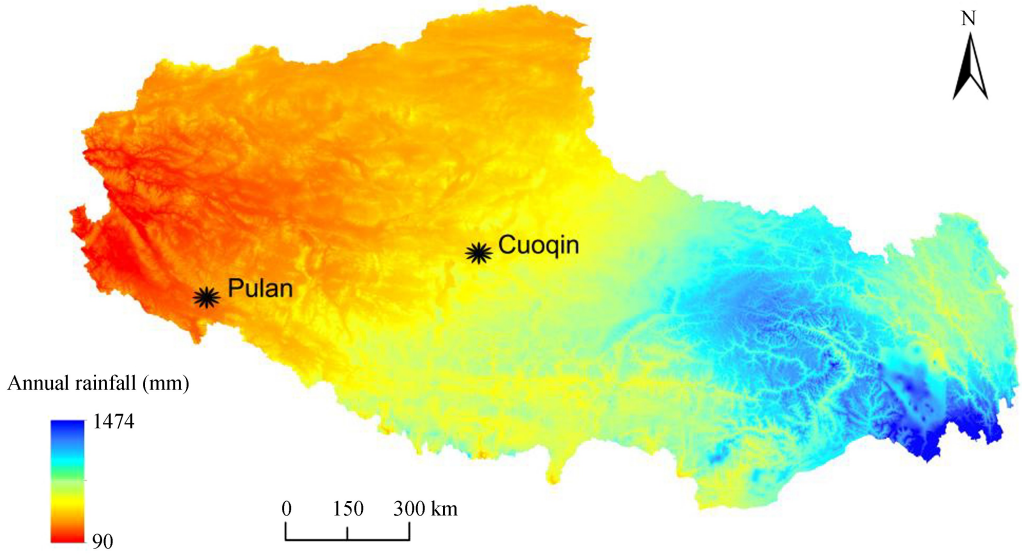
1.1 Seed collection and cultivation

The mature *S. purpurea* seeds were collected in August 2013 at the time of seed release from two populations of western Pulan and eastern Cuoqin respectively on the Tibetan Plateau (Fig. 1). After brought back to laboratory, the seeds were dried under a constant condition of 15 ℃, 15% air humidity for one week.

The full seeds of two populations were picked out randomly and the seed awns were removed. Then the seeds were sown in flowerpots [diameter = 6.8–9.4 cm (from bottom to top), height = 8 cm] with equal weight of humus soil, watering to keep the soil sufficiently moist. Each pot contained thirty seeds. The pots were placed in a greenhouse under 12-h-light/12-h-dark, 28/20 ℃ and a relative humidity of 40%–60%. For each population, leaves of ten-pot seedlings were collected and grinded for proline, MDA, antioxidant enzyme activity measurement and RNA extraction. 3 one-pot seedlings were used to analyze change of morphology, RWC in leaf and chlorophyll fluorescence respectively. Three leaves in one pot were selected randomly for H_2O_2 and O_2^- detection. Three independent replications were carried out for all sample measurements.

1.2 Drought treatment

Before drought treatment, the seedlings grew for another three weeks watered every day uniformly. When the plants reached the 3-leaf seedling stage (Liu *et al.*, 2013), the plants were withheld water for two weeks until the plants of one population showed apparent death. Then the plants were rewatered for



Site	Longitude and latitude	Altitude (m)
Pulan	E 81°21′39″ N 30°42′57″	4639
Cuoqin	E 85°05′42″ N 31°03′06″	4794

Fig. 1 Geographical information and location on the precipitation distribution map of the two seed collection sites

another two weeks; after recovery, the plant mortality for each population was counted. The leaves of plants under drought for 0 d and 14 d and rehydration for 14 d were sampled for the following measurement and analysis.

1.3 Water content measurement

The relative water content (RWC) of the leaves was determined as: RWC = [Fresh weight (FW) − Drought weight (DW)]/(Turgid weight-DW). To measure turgid weight, leaves were kept in distilled water in darkness at 4 ℃ to minimize respiration losses until they reached a constant weight (Rivero *et al.*, 2007). DW of the plants was obtained after 48 h at 70 ℃ in an air oven (Rivero *et al.*, 2007).

1.4 Analysis of chlorophyll fluorescence

Chlorophyll fluorescence was analyzed as previously described (Bai *et al.*, 2011), with a pulse-amplitude modulation chlorophyll fluorometer (Heinz Walz GmbH, Effeltrich, Germany). Briefly, at the time of sampling, *S. purpurea* seedlings were dark-adapted for 30 min to measure the maximum quantum yield of photosystem Ⅱ (PSⅡ; F_v/F_m), and F_v/F_m was determined for each sample by analyzing a whole pot plant. The maximum fluorescence (F_m) was recorded by a 0.8 s pulsed light at 4 000 μmol s^{-1} m^{-2}, and minimal fluorescence (F_o) was recorded during the weak measuring pulses.

1.5 Proline and malondialdehyde content measurement

Proline content was measured as previously reported (Bates *et al.*, 1973), with some modification. Approximately 0.2 g of fresh leaves was homogenized in 10 mL of 3% aqueous sulphosalicylic acid, and the homogenate was centrifuged at 2 000 × *g* for 10 min. Then, 2 mL of the extract were reacted with 2 mL of acidic-ninhydrine and 2 mL of glacial acetic acid for 1 h in boiling water; after this, the reaction terminated in an ice bath. The reaction mixture was extracted with 4 mL toluene, mixed vigorously with a test tube stirrer for 15 − 20 sec. The chromophore containing toluene was aspirated from the aqueous phase, warmed to room temperature and the absorbance read at 520 nm using toluene for a blank. The proline concentration was determined from a standard curve and calculated as mg g^{-1} DW.

The malondialdehyde (MDA) content was de-

termined as described previously (Li *et al.*, 2014). Approximately 0.5 g of fresh leaves were homogenized in 10 mL of 10% trichloroacetic acid (TCA) and centrifuged at 12 000 × *g* for 10 min. Then, 2 mL of 0.6% thiobarbituric acid in 10% TCA were added to an aliquot of 2 mL of the supernatant. The mixture was heated in boiling water for 30 min and then quickly cooled in an ice bath. After centrifugation at 10 000 × *g* for 10 min, the absorbance of the supernatant at 450, 532, and 600 nm was determined. The MDA concentration was expressed as nmol g^{-1} DW.

1.6 *In situ* H_2O_2 and O_2^- detection

The *in situ* detection of H_2O_2 and O_2^- were performed using a previously reported method with some modifications (Able, 2003). The amount of H_2O_2 and O_2^- was detected with 1 mg mL^{-1} of diaminobenzidine (DAB) and 10^{-2} M nitro-blue tetrazolium (NBT) respectively. Leaves were vacuum-infiltrated in 10 mL solution for 2 h and then cleared in boiling ethanol (95%) for 10 min. Then, samples were stored and examined in 95% ethanol.

1.7 Antioxidant enzyme activity assays

The activities of superoxide dismutase (SOD; EC1.15.1.1), catalase (CAT; EC1.11.1.6) and ascorbate peroxidase (APX; EC1.11.1.11) were determined as previously described (Nakano and Asada, 1981; Jiang and Zhang, 2001). Approximately 1 g of leaves collected from treated *S. purpurea* seedlings were homogenized in 10 mL extraction buffer (50 mmol · L^{-1} sodium phosphate pH 7.0, 1 mmol · L^{-1} EDTA, 1 mmol · L^{-1} DTT, 1 mmol · L^{-1} GSH, 5 mmol · L^{-1} $MgCl_2 \cdot 6H_2O$, 1% PVP-40 and 20% glycerin). The homogenates were centrifuged at 12 000 × *g* for 10 min at 4 ℃, and total soluble protein content of the supernatants was measured by the Bradford method (Barbosa *et al.*, 2009).

1.8 RNA extraction and RT-PCR analysis

Total RNA was extracted from the leaves of different samples using TRIzol reagent (Invitrogen) and treated with RNase-free DNAse (Takara). The RNA concentration was determined using a Nanodrop 1 000 (Thermo Scientific product, USA). MMLV reverse transcriptase (Promega) was used to synthesize the cDNAs. The alternatively spliced fragments were amplified from the cDNA using primers listed in Table 1, which were designed based on the transcriptomic sequencing results. The amplification was performed under conditions as follows: 94 ℃, 4 min; 94 ℃, 30 s; 52–58 ℃, 30 s; 72 ℃, 30 s; 28–35 cycles; 72 ℃, 10 min (Table 1). The amplification products were separated by electrophoresis on 2% agarose gels.

1.9 Data analysis

Statistical analyses were performed using the statistical Software Package for Social Science (SPSS) version 18.0. One-way ANOVA for all variables was used for testing the treatment differences. Differences were considered to be significant at the $P < 0.05$.

2 Results

2.1 The phenotypic difference between plants from two sites

After exposure to drought stress for 14 days under the same conditions, the plants of Cuoqin showed obvious wilting compared with those of Pulan (Fig. 2A). Some of the plants died after another 14-d rehydration with significant divergence between plants of two sites. Actually, 16.2% of the seedlings from Pulan were dead while mortality rate of Cuoqin reached to 78.2% (Fig. 2B). The phenotypic results fully demonstrated that there was difference between the plants from two sites in response to drought stress.

2.2 The changes of physiological status of the plants

The RWC of the plants from both Pulan and Cuoqin had high values (83.9% and 83.3% respectively) before drought treatment and dropped to dissimilarly low levels (32.0% and 13.6% respectively) after drought stress for 14 d, with obvious difference between two sites (Fig. 2C). After rewatered, the RWC of plants from Pulan returned to a relatively high level (69.1%) while that of Cuoqin still had a very low value (35.1%) (Fig. 2C), indicating that maybe the water absorption system of plants from Cuoqin was destroyed by the stress.

Table 1 The information of genes related to drought stress used for RT-PCR

Gene name	GenBank accession number	Primer F (5′-3′)	Primer R (5′-3′)	T_m /℃	PCR cycles
Actin1	KM216249	GCTGGATTCTGGAGATGGTGTC	TTACTCATTCACCACTACGGCTG	52	28 and 35
APX1	KM201379	CACGAGGAAGAATACACCC	GCATAAAGCTCCACATAGC	50	30
APX2	KM201380	CCTTCGGCACCATGAAGTG	GCCTCAGCATAGTCAGCAAA	54	30
CAT	KM201378	CACGCCTTCAAGCCCAAC	ACGAACCTGTCCTGCCTGTC	56	30
DREB1	KM201387	GGACGTTCCCAACTGCTC	CGGTTCACCTTCTATCGG	52	32
DREB3	KM201388	GTTGGCTCGTACTAACTTCC	ATCATCGGTTCACCTTCTAT	56	32
P5CS	KM201383	TCATAACTGGCGTCATTC	TTGTCCACTCCCTCGTAG	50	30
PIP1	KM201389	TCCGAGGACAAGGACTACAAGG	GGACGAAGGTGCCGATGAT	56	30
PIP2	KM201390	CATTGACTTCGAGGAGCTGACC	GGTGCTTGTACCCGATGACG	56	30
SOD1	KM201381	CTTATTTGAGCAAGAGGG	CTGATGGCATTTCGTGTA	48	30
SOD2	KM201382	GAAGCACCACGCCACCTA	GCTCCCAGACATCAATCCC	54	30
WRKY4	KM201385	AGCCTTCACTGAGCCTTGACC	CCTTCTGCCCGTACTTCCG	58	30
WRKY11	KM201386	CCCTCCCGTTTCTTCTCCTCCT	GGGCTTCTGTCCGTACTTGCG	58	30
WRKY17	KM201384	CAGAAGCACGTTAAGGGA	GAGGCTCACGGTTAGGTT	52	30

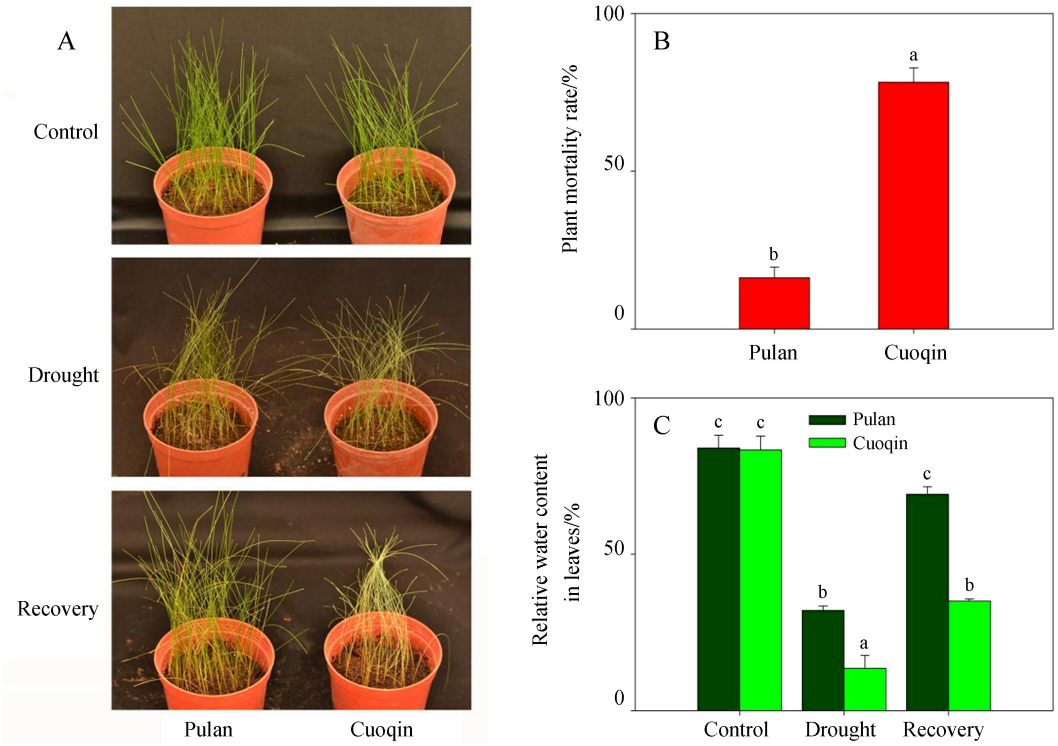


Fig. 2 Changes in plant phenotype and relative water content of leaves during the process of drought treatment and subsequent recovery of *Stipa purpurea* from two sites. A: The change of plant phenotype of *Stipa purpurea* from two sites; B: The change of plant mortality rate of *Stipa purpurea* from two sites; C: The change in relative water content of leaves in *Stipa purpurea* from two sites. Error bars indicate SE. Means denoted by different letters were significantly different ($P < 0.05$) (B & C)

The chlorophyll fluorescence was diversely influenced in the course of drought stress and subsequent recovery. The F_v/F_m of plants from Pulan just changed from 0.86 to 0.52 during the drought treatment (Fig. 3); nevertheless, the F_v/F_m reduced from 0.86 to 0.05 in the plants from Cuoqin (Fig. 3). In the recovery stage, the F_v/F_m returned to an average value of 0.81 and 0.20 respectively in the plants of Pulan and Cuoqin (Fig. 3), suggesting the different influence degree under drought stress.

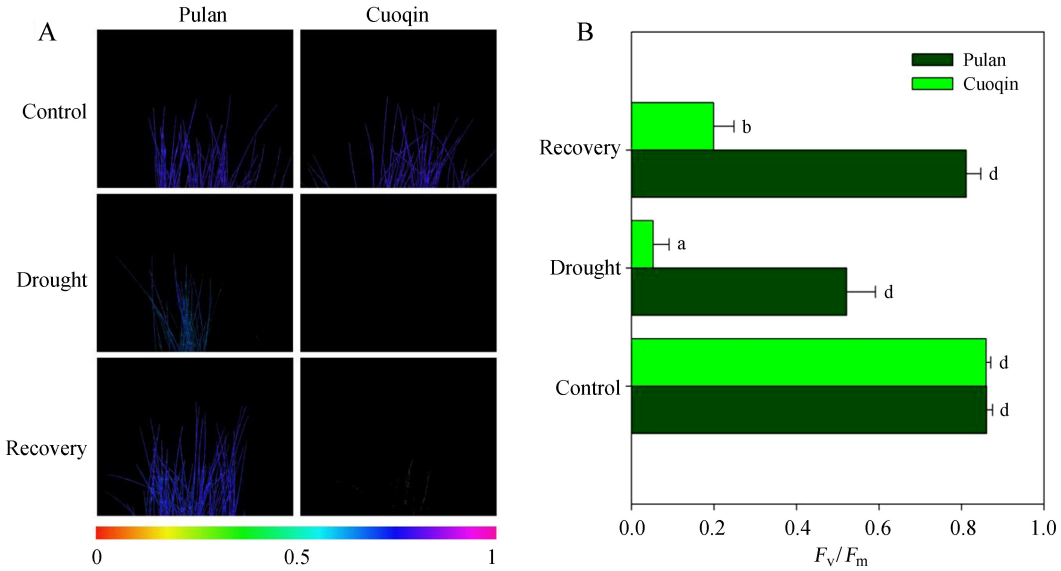


Fig. 3　Effects of drought stress and subsequent recovery on leaf photosynthesis in *Stipa purpurea* from two sites. A: F_v/F_m images. The pseudocolor code depicted at the bottom of the image ranges from 0 (red) to 1.0 (purple). The experiment was replicated three times with similar results. One representative result was shown; B: Average F_v/F_m values. F_v/F_m was determined for whole plants. Error bars indicate SE. Means denoted by different letters were significantly different ($P < 0.05$) (B)

2.3　The changes of proline and MDA content

The proline content of plants from both Pulan and Cuoqin kept at a relatively low level before drought stress (1.51 and 1.52 mg g^{-1} DW respectively) (Fig. 4A). After treated by drought stress for two weeks, to our surprise, the proline content of plants from Pulan was drastically induced up to 10.11 mg g^{-1} while it rose up to 6.07 mg g^{-1} in plants from Cuoqin, which were 5.7 and 3.0 fold respectively more than the corresponding controls (Fig. 4A). In the subsequent recovery, the proline content in both plants recovered by certain degree, but they were still higher than the controls (1.96 and 2.68 mg g^{-1} respectively) (Fig. 4A).

The MDA content was diversely influenced in the course of drought stress and subsequent recovery. It changed from 4.18 to 17.09 nmol g^{-1} DW during the drought stress in the plants from Pulan (Fig. 4B), nevertheless, it increased from 4.07 to 22.83 nmol g^{-1} DW in the plants from Cuoqin (Fig. 4B). In the recovery stage, the MDA content returned to the control level in the plants from Pulan (3.34 nmol g^{-1} DW), but it remained at a higher level than the control in the plants from Cuoqin (12.86 nmol g^{-1} DW) (Fig. 4B).

2.4　The accumulation of H_2O_2 and O_2^-

Both the H_2O_2 and O_2^- in plants from two sites were accumulated under exposure to drought stress and dropped to a low level after rewatered, but they both showed different change degree between plants of two sites (Fig. 4C). Both H_2O_2 and O_2^- were induced to a much lower level and returned to be more similar with the control in the plants from Pulan compared with those from Cuoqin (Fig. 4C). The results were consistent with the MDA content, which reflected the oxidation degree of cell membrane by the excessive ROS accumulation.

2.5　The dynamic of antioxidant enzyme activities

The SOD activity, as well as APX activity, were induced significantly under drought stress and returned to the control level roughly in the plants from Pulan (Fig. 5A). However, both SOD and APX activities did not increase but decreased slightly after drought treatment in plants from Cuoqin (Fig. 5A); when the plants werewatered again, the activities of both the two antioxidant enzymes contin-

ued to reduce to a lower level compared with the control (Fig. 5A). The CAT activity also rose greatly in plants from Pulan when the plants were treated by drought stress and went back to the control level after rehydration treatment, while it just increased slightly under drought stress and the value reduced to the control below after rehydration in the plants from Cuoqin (Fig. 5A). The results indicated that the antioxidant enzyme system in plants from Cuoqin was inefficient and might be partly destroyed in the course of drought stress.

2.6 The expression changes of genes related to drought stress

To investigate the expression patterns and functions of genes in response to drought, RT-PCR was performed for several drought-related genes. The results indicated that different genes showed various expression changes pattern with two common points (Fig. 5B). On one hand, nearly all genes' expressions were induced by different degrees under drought treatment in both sites of plants (Fig. 5B). On the other hand, most genes had higher expressions in plants from of Pulan compared with those from Cuoqin after exposure to drought stress (Fig. 5B). More specifically, two SOD genes (SOD1, SOD2) and one CAT gene were all induced obviously under drought stress and returned to the control levels after rewatering in plants from Pulan, but they showed

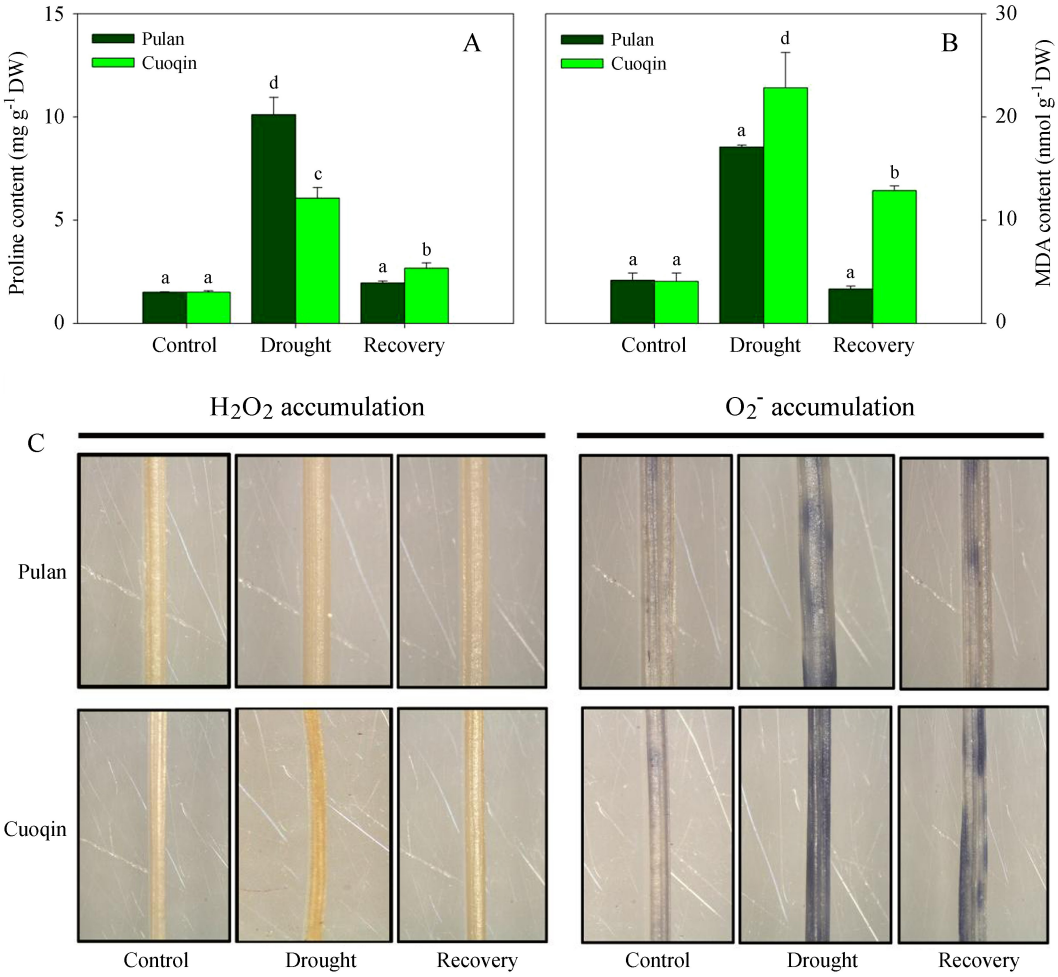


Fig. 4 Accumulation of proline, malondialdehyde (MDA) and reactive oxygen species (ROS) (H_2O_2 and O_2^-) in *Stipa purpurea* from two sites during the process of drought treatment and subsequent recovery. A: Proline content changes in *Stipa purpurea* from two sites; B: MDA content changes in *Stipa purpurea* from two sites; C: *In situ* detection of changes in leaf H_2O_2 and O_2^- levels of *Stipa purpurea* from two sites. Error bars indicate SE. Means denoted by different letters were significantly different ($P < 0.05$) (A and B)

slight induction under drought stress and dropped below the control levels after watering again in plants from Cuoqin (Fig. 5B). Two APX genes (APX1, APX2) were also induced under drought stress and went back to the control levels in plants from Pulan; on the contrary, expression of the two genes did not increase but decrease after drought treatment in plants from Cuoqin (Fig. 5B). The delta-1-pyrroline-5-carboxylate synthase-like (P5CS) gene showed obviously high expression in both plants from Pulan and Cuoqin (Fig. 5B). Three WRKY family genes (*WRKY4*, *WRKY11* and *WRKY17*) had similar expression changes in the plants from the same site; nevertheless, they showed better induction and recovery in plants from Pulan than those of Cuoqin (Fig. 5B). Interestingly, the similar results were found in two dehydration responsive element binding protein (DREB) family genes (DREB1 and DREB3) (Fig. 5B). Two aquaporin genes (*PIP1* and *PIP2*), which belong to the class of plasma membrane intrinsic proteins (PIPs), also showed higher expressions in plants from Pulan compared with those from Cuoqin after exposure to drought stress (Fig. 5B).

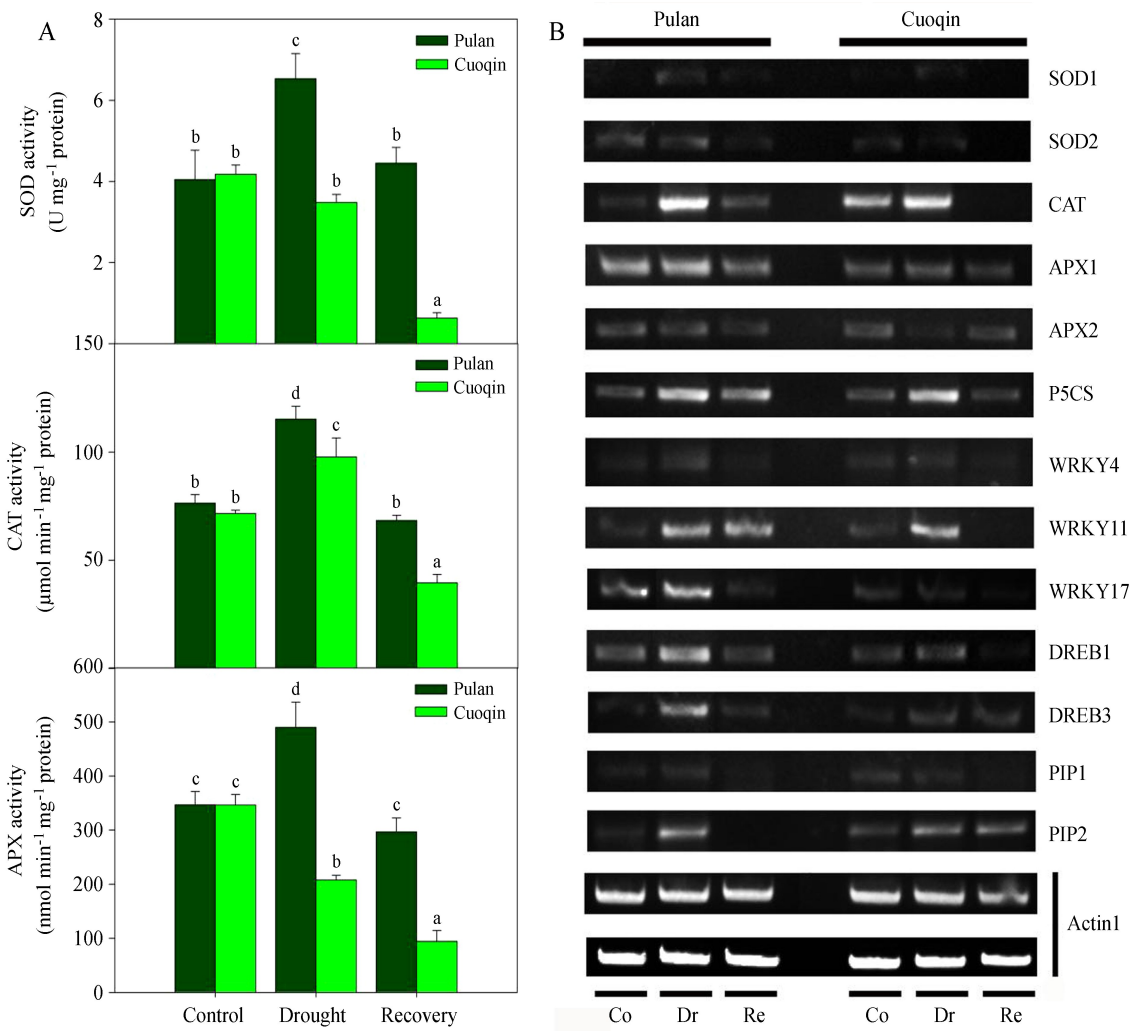


Fig. 5　Changes in antioxidant enzyme activities and drought-related genes expression in *Stipa purpurea* from two sites during the process of drought treatment and subsequent recovery. A: The changes of antioxidant enzyme activities in *Stipa purpurea* from two sites; B: The changes of drought-related genes expression in *Stipa purpurea* from two sites. Actin was included as a cDNA loading control. The abbreviations Co, Dr and Re at the bottom represent Control, Drought and Recovery respectively. Error bars indicate SE. Means denoted by different letters were significantly different ($P < 0.05$) (A)

3 Discussion

Intraspecific variation in drought response has been observed from a lot of species under different growth conditions (Walker and Miller, 1986; Sandquist and Ehleringer, 1997, 1998; Beikircher and Mayr, 2009; Lu *et al.*, 2009; Hamanishi *et al.*, 2010). These findings show the effects of environmental heterogeneity on the modification and acclimation in plant characteristics. As *S. purpurea* is distributed along a representative precipitation gradient, the characteristics of response to water shortage or excess also may exhibit significant differences in different populations, with a hypothesis that plants from more arid area may possess greater drought tolerance. In the present study, the results of morphological and physiological status in the course of drought treatment and subsequent recovery fully suggested that the seedlings from Pulan had stronger resistance to the same degree of drought stress than those from Cuoqin. That was to say, *S. purpurea* from more arid area had stronger resistivity to drought stress; therefore, the results implied that the intraspecific difference of response to drought presented genetic effects.

Low leaf RWC after drought caused obvious withering and chlorsis in plants from Cuoqin that suffered from greater mortality as a result, indicating that plants from Cuoqin had been exceeded physiological drought limit. Water shortage can induce stomatal closure, which will influence photosynthesis process directly. The photosynthetic apparatus associated with PS II is very sensitive to drought and various other stresses (Yordanov *et al.*, 1999; Mihailova *et al.*, 2011), which usually reduces chlorophyll fluorescence. In the present study, the chlorophyll fluorescence (F_v/F_m) of plants from Pulan dropped less and recovered more than those from Cuoqin, indicating that plants from Cuoqin were more susceptible to drought and more difficult to recover from the stress. The decline of F_v/F_m often resulted from a non-radiative process of thermally dissipating absorbed light, which played a central role in leaf photoprotection under drought (Chaves *et al.*, 2003). If excess energy from absorbed light was not coped with by either photosynthesis or photorespiration and the thermal dissipation, the production of highly reactive molecules, generated within the chloroplast, could be exacerbated to cause oxidative damage to the pthotosynthetic apparatus (Smirnoff, 1998; Niyogi, 1999).

Oxidative stress is a state of damage caused by ROS, which consists of H_2O_2, O_2^-, OH and $^1O_2$ (Chaves *et al.*, 2003). Under normal conditions, ROS are scavenged by antioxidant molecules and enzymes that are located in different cell compartments, which maintain ROS homeostasis in plants (Li *et al.*, 2014). In this work, we tested the ROS (H_2O_2 & O_2^-) accumulation in conjunction with the antioxidant enzyme activities (SOD, CAT & APX) and the expression of their corresponding regulatory genes. Smaller amounts of H_2O_2 and O_2^- were observed both after drought treatment and subsequent recovery in plants from Pulan, the reason could be explained by the higher activities of antioxidant enzymes. For the plants from Cuoqin, because the lower antioxidant enzymes activities had poor efficiency to remove the excessive ROS, so the plants failed to maintain the normal physiological state and even died heavily due to the oxidative damage partly. Interestingly, the changes of antioxidant enzyme activities were very similar to the expression results of the corresponding genes, implying that the divergences between plants from two sites were internal genetic properties. When the ROS dynamic equilibrium is broken, a series of oxidation products will be generated, such as MDA, a commonly used index that reflected grades of cellular oxidation (Li *et al.*, 2014). In the present study, less content of MDA in the seedlings of *S. purpurea* from Pulan also indirectly illustrated their better capability to cope with drought stress.

Osmotic adjustment is considered a crucial process in plant adaptation to drought because of its role in sustaining tissue metabolic activity and enabling re-

growth upon rewetting (Morgan, 1984). Proline, the synthesis of which is mainly regulated by P5CS gene (Hare *et al.*, 1999), is one of the most studied compatible solutes in osmotic adjustment. Proline helps to stabilize macromolecules, protect enzymes, and store carbon and nitrogen for use during stress regimes in plants (Ashraf and Foolad, 2007). Our results showed that the proline production could be rapidly and substantially induced under drought stress in *S. purpurea*, and the accumulation amount was much more in plants from Pulan, which had better drought tolerance. Interestingly, the change trend of proline content was consistent with the expression of P5CS gene in the process. This suggested that extremely efficient start of regulatory genes, including P5CS, and accumulation of proline had significant roles in response and resistance to drought in *S. purpurea*.

Transcription factors (TFs) have been demonstrated to play important roles at various levels in the signaling web to enable plants to deal with water-stress/drought (Tripathi *et al.*, 2014). WRKY TFs, both positive and negative regulators of gene expression (Eulgem and Somssich, 2007), have been reported to play pivotal roles in regulating many stress reactions in plants (Chen *et al.*, 2012; Rushton *et al.*, 2012). Specially, overexpression of several WRKY TFs has been proved to enhance drought and some other stresses tolerance in different species (Zhou *et al.*, 2008; Qiu and Yu, 2009; Wu *et al.*, 2009; Moon *et al.*, 2014). In the study, three WRKY TFs were all induced under drought stress and had higher expression in *S. purpurea* from Pulan rather than Cuoqin, implying their important functions in response to drought stress in *S. purpurea* and the role of greater expression in strengthening drought tolerance in plants from Pulan. DREBs, which control stress-inducible gene expression in the abscisic acid (ABA)-independent pathway (Yamaguchi-Shinozaki and Shinozaki, 2006), are another class of TFs that have been reported to play an important role in plant response to water stress in many species (Liu *et al.*, 2013). The expression changes of two DREB TFs in our study also indicated their significant functions in response to drought stress and the role of greater expression in driving stronger drought tolerance in *S. purpurea* from Pulan.

The regulation of aquaporins is one important response of plant cells to water stress (Luu *et al.*, 2007), which tightly control the transcellular water movement by their amount and activity (Chaumont and Tyerman, 2014). PIPs are one of the five subgroups of aquaporins (Johanson *et al.*, 2001; Danielson and Johanson, 2008), which can be divided into two major groups in plants, PIP1 and PIP2, based on their sequences and water-channel activity (Kelly *et al.*, 2014). Sufficient evidence has demonstrated that overexpression of PIPs significantly enhanced the rates of growth, transpiration and ptotosynthesis in Arabidopsis and tomato plants (Aharon *et al.*, 2003; Flexas *et al.*, 2006; Sade *et al.*, 2010), and the antisense suppression usually had the opposite effects (Siefriz *et al.*, 2002; Uehlein *et al.*, 2003; Siefritz *et al.*, 2004). In addition, a putative aquaporin gene *VfPIP1*, isolated from *Vicia faba* leaf epidermis, was found to be induced in expression by ABA and polyethylene glycol 6 000 and its expression might improve drought resistance of the transgenic plants (Cui *et al.*, 2008). Like the previous studies, two PIPs genes, PIP1 and PIP2, were both induced in expression in *S. purpurea* under drought stress; furthermore, their expression were much higher in plants from Pulan, which displayed greater drought tolerance in the study. The results once again indicated that PIPs could improve the drought resistance of plants.

Above all, our study indicated that different *S. purpurea* populations had variant responses to drought stress throughout long-term adaptation and evolution. Rapid and efficient start of response and protection mechanism at physiological and molecular levels might be the foundation of stronger resistance to drought stress for *S. purpurea*. The results indicated the differential responses to climate change among

different *S. purpurea* populations. The study helps us to understand more about the adaptation and evolution of alpine plants in the natural habitat on the Tibetan Plateau under global climate change.

References:

Able AJ, 2003. Role of reactive oxygen species in the response of barley to necrotrophic pathogens [J]. *Protoplasma*, **221**: 137—143

Aharon R, Shahak Y, Wininger S *et al.*, 2003. Overexpression of a plasma membrane aquaporin in transgenic tobacco improves plant vigor under favorable growth conditions but not under drought or salt stress [J]. *The Plant Cell*, **15**: 439—447

Aranjuelo I, Molero G, Erice G *et al.*, 2011. Plant physiology and proteomics reveals the leaf response to drought in alfalfa (*Medicago sativa* L.) [J]. *Journal of Experimental Botany*, **62**: 111—12

Ashraf M, Foolad MR, 2007. Roles of glycine betaine and proline in improving plant abiotic stress resistance [J]. *Environmental and Experimental Botany*, **59**: 206—216

Bai XG, Yang LM, Yang YQ *et al.*, 2011. Deciphering the protective role of nitric oxide against salt stress at the physiological and proteomic levels in maize [J]. *Journal of Proteome Research*, **10**: 4349—4364

Barbosa H, Slater NKH, Marcos JC, 2009. Protein quantification in the presence of poly (ethylene glycol) and dextran using the Bradford method [J]. *Analytical Biochemistry*, **395**: 108—110

Bates LS, Waldren RP, Teare ID, 1973. Rapid determination of free proline for water-stress studies [J]. *Plant and Soil*, **39**: 205—207

Beikircher B, Mayr S, 2009. Intraspecific differences in drought tolerance and acclimation in hydraulics of *Ligustrum vulgare* and *Viburnum lantana* [J]. *Tree Physiology*, **29**: 765—775

Bothe O, Fraedrich K, Zhu XH, 2010. The large-scale circulations and summer drought and wetness on the Tibetan plateau [J]. *International Journal of Climatology*, **30**: 844—855

Bothe O, Fraedrich K, Zhu XH, 2011. Large-scale circulations and Tibetan Plateau summer drought and wetness in a high-resolution climate model [J]. *International Journal of Climatology*, **31**: 832—846

Carlyle C, Fraser LH, Turkington R, 2014. Response of grassland biomass production to simulated climate change and clipping along an elevation gradient [J]. *Oecologia*, **174**: 1065—1073

Catala R, Ouyang J, Abreu IA *et al.*, 2007. The Arabidopsis E3 SUMO ligase SIZ1 regulates plant growth and drought responses [J]. *The Plant Cell*, **19**: 2952—296

Chaumont F, Tyerman SD, 2014. Aquaporins: highly regulated channels controlling plant water relations [J]. *Plant Physiology*, **164**: 1600—1618

Chaves MM, Maroco JP, Pereira JS, 2003. Understanding plant responses to drought-from genes to the whole plant [J]. *Functional Plant Biology*, **30**: 239—264

Chen LG, Song Y, Li SJ *et al.*, 2012. The role of WRKY transcription factors in plant abiotic stresses [J]. *Biochimica Et Biophysica Acta-Gene Regulatory Mechanisms*, **1819**: 120—128

Cui XH, Hao FS, Chen H *et al.*, 2008. Expression of the *Vicia faba VfPIP1* gene in *Arabidopsis thaliana* plants improves their drought resistance [J]. *Journal of Plant Research*, **121**: 207—214

Danielson JAH, Johanson U, 2008. Unexpected complexity of the Aquaporin gene family in the moss *Physcomitrella patens* [J]. *BMC Plant Biology*, **8**: 45

Fang KY, Wilmking M, Davi N *et al.*, 2014. An ensemble weighting approach for dendroclimatology: drought reconstructions for the northeastern Tibetan Plateau [J]. *PloS One*, **9**: e86689

Eulgem T, Somssich IE, 2007. Networks of WRKY transcription factors in defense signaling [J]. *Current Opinion in Plant Biology*, **10**: 366—371

Flexas J, Ribas-Carbo M, Hanson DT *et al.*, 2006. Tobacco aquaporin *NtAQP1* is involved in mesophyll conductance to CO2 *in vivo* [J]. *The Plant Journal*, **48**: 427—439

Fordyce JA, 2006. The evolutionary consequences of ecological interactions mediated through phenotypic plasticity [J]. *Journal of Experimental Biology*, **209**: 2377—2383

Fu QS, Yang RC, Wang HS *et al.*, 2013. Leaf morphological and ultrastructural performance of eggplant (*Solanum melongena* L.) in response to water stress [J]. *Photosynthetica*, **51**: 109—114

Gou XH, Chen FH, Jacoby G *et al.*, 2007. Rapid tree growth with respect to the last 400 years in response to climate warming, northeastern Tibetan Plateau [J]. *International Journal of Climatology*, **27**: 1497—1503

Gou XH, Chen FH, Yang MX *et al.*, 2006. A comparison of tree-ring records and glacier variations over the past 700 years, northeastern Tibetan Plateau [J]. *Annals of Glaciology*, **43**: 86—90

Hamanishi ET, Raj S, Wilkins O *et al.*, 2010. Intraspecific variation in the *Populus balsamifera* drought transcriptome [J]. *Plant Cell and Environment*, **33**: 1742—1755

Hare PD, Cress WA, van Staden J, 1999. Proline synthesis and degradation: a model system for elucidating stress-related signal transduction [J]. *Journal of Experimental Botany*, **50**: 413—434

Hu MY, Zhang L, Luo TX *et al.*, 2012. Variations in leaf functional traits of *Stipa purpurea* along a rainfall gradient in Xizang, China [J]. *Chinese Journal of Plant Ecology*, **36**: 136—143

Huang GT, Ma SL, Bai LP *et al.*, 2012. Signal transduction during cold, salt, and drought stresses in plants [J]. *Molecular Biology Reports*, **39**: 969—987

Jiang MY, Zhang JH, 2001. Effect of abscisic acid on active oxygen species, antioxidative defence system and oxidative damage in leaves of maize seedlings [J]. *Plant and Cell Physiology*, **42**:

1265—1273

Johanson U, Karlsso, M, Johansson I *et al.*, 2001. The complete set of genes encoding major intrinsic proteins in arabidopsis provides a framework for a new nomenclature for major intrinsic proteins in plants [J]. *Plant Physiology*, **126**: 1358—1369

Kelly G, Sade N, Attia Z *et al.*, 2014. Relationship between Hexokinase and the aquaporin PIP1 in the regulation of photosynthesis and plant growth [J]. *PloS One*, **9**: e87888

Klein JA, Harte J, Zhao XQ, 2004. Experimental warming causes large and rapid species loss, dampened by simulated grazing, on the Tibetan Plateau [J]. *Ecology Letters*, **7**: 1170—1179

Li X, Yang YQ, Ma L *et al.*, 2014. Comparative proteomics analyses of *Kobresia pygmaea* adaptation to environment along an elevational gradient on the central Tibetan Plateau [J]. *PloS One*, **9**: e98410

Liu S, Wang X, Wang H *et al.*, 2013. Genome-wide analysis of *ZmDREB* genes and their association with natural variation in drought tolerance at seedling stage of *Zea mays* L [J]. *PloS Genetics*, **9**: e1003790

Lu YW, Duan BL, Zhang XL *et al.*, 2009. Intraspecific variation in drought response of *Populus cathayana* grown under ambient and enhanced UV-B radiation [J]. *Annals of Forest Science*, **66**: 613

Luu D, Boursiac Y, Chen S *et al.*, 2007. Regulation of plant aquaporins in response to water stress [J]. *Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology*, **146**: S152—S152

Mihailova G, Petkova S, Buchel C *et al.*, 2011. Desiccation of the resurrection plant *Haberlea rhodopensis* at high temperature [J]. *Photosynthesis Research*, **108**: 5—13

Moon SJ, Han SY, Kim DY *et al.*, 2014. Ectopic expression of *CaWRKY1*, a pepper transcription factor, enhances drought tolerance in transgenic potato plants [J]. *Journal of Plant Biology*, **57**: 198—207

Moore JP, Vicre-Gibouin M, Farrant JM *et al.*, 2008. Adaptations of higher plant cell walls to water loss: drought vs desiccation [J]. *Physiologia Plantarum*, **134**: 237—245

Morgan JM, 1984. Osmoregulation and water-stress in higher-plants [J]. *Annual Review of Plant Physiology and Plant Molecular Biology*, **35**: 299—319

Nakano Y, Asada K, 1981. Hydrogen-peroxide is scavenged by ascorbate-specific peroxidase in spinach-chloroplasts [J]. *Plant and Cell Physiology*, **22**: 867—880

Nelson DE, Repetti PP, Adams TR *et al.*, 2007. Plant nuclear factor Y (NF-Y) B subunits confer drought tolerance and lead to improved corn yields on water-limited acres [J]. *Proceedings of the National Academy of Sciences of the United States of America*, **104**: 16450—16455

Ni J, 2000. A simulation of biomes on the Tibetan Plateau and their responses to global climate change [J]. *Mountain Research and Development*, **20**: 80—89

Nicotra AB, Davidson A, 2010. Adaptive phenotypic plasticity and plant water use [J]. *Functional Plant Biology*, **37**: 117—127

Niyogi KK, 1999. Photoprotection revisited: Genetic and molecular approaches [J]. *Annual Review of Plant Physiology and Plant Molecular Biology*, **50**: 333—359

Qiu YP, Yu DQ, 2009. Over-expression of the stress-induced *OsWRKY45* enhances disease resistance and drought tolerance in Arabidopsis [J]. *Environmental and Experimental Botany*, **65**: 35—47

Rivero RM, Kojima M, Gepstein A *et al.*, 2007. Delayed leaf senescence induces extreme drought tolerance in a flowering plant [J]. *Proceedings of the National Academy of Sciences of the United States of America*, **104**: 19631—19636

Rushton DL, Tripathi P, Rabara RC *et al.*, 2012. WRKY transcription factors: key components in abscisic acid signaling [J]. *Plant Biotechnology Journal*, **10**: 2—11

Sade N, Gebretsadik M, Seligmann R *et al.*, 2010. The Role of Tobacco Aquaporin1 in Improving Water Use Efficiency, Hydraulic Conductivity, and Yield Production Under Salt Stress [J]. *Plant Physiology*, **152**: 245—254

Sandquist DR, Ehleringer JR, 1997. Intraspecific variation of leaf pubescence and drought response in *Encelia farinosa* associated with contrasting desert environments [J]. *New Phytologist*, **135**: 635—644

Sandquist DR, Ehleringer JR, 1998. Intraspecific variation of drought adaptation in brittlebush: leaf pubescence and timing of leaf loss vary with rainfall [J]. *Oecologia*, **113**: 162—169

Scandalios JG, 1993. Oxygen stress and superoxide dismutase [J]. *Plant Physiology*, **101**: 7—12

Shen CM, Liu KB, Morrill C *et al.*, 2008. Ecotone shift and major droughts during the mid-late holocene in the central Tibetan Plateau [J]. *Ecology*, **89**: 1079—1088

Siefritz F, Otto B, Bienert GP *et al.*, 2004. The plasma membrane aquaporin *NtAQP1* is a key component of the leaf unfolding mechanism in tobacco [J]. *The Plant Journal*, **37**: 147—155

Siefritz F, Tyree MT, Lovisolo C *et al.*, 2002. PIP1 plasma membrane aquaporins in tobacco: From cellular effects to function in plants [J]. *The Plant Cell*, **14**: 869—876

Smirnoff N, 1998. Plant resistance to environmental stress [J]. *Current Opinion in Biotechnology*, **9**: 214—219

Sun JY, Liu Y, 2013. Drought variations in the middle Qilian Mountains, northeast Tibetan Plateau, over the last 450 Years as reconstructed from tree rings [J]. *Dendrochronologia*, **31**: 279—285

Sun XL, Sun MZ, Luo X *et al.*, 2013. A Glycine soja ABA-responsive receptor-like cytoplasmic kinase, *GsRLCK*, positively controls plant tolerance to salt and drought stresses [J]. *Planta*, **237**: 1527—1545

Terzi R, Saruhan Guler N, Kutlu Caliskan N *et al.*, 2013. Lignifica-

tion response for rolled leaves of Ctenanthe setosa under long-term drought stress [J]. *Turkish Journal of Biology*, **37**: 614—619

Tripathi P, Rabara RC, Rushton PJ, 2014. A systems biology perspective on the role of WRKY transcription factors in drought responses in plants [J]. *Planta*, **239**: 255—266

Turgut R, Kadioglu A, 1998. The effect of drought, temperature and irradiation on leaf rolling in *Ctenanthe setosa* [J]. *Biologia Plantarum*, **41**: 629—633

Uehlein N, Lovisolo C, Siefritz F *et al.*, 2003. The tobacco aquaporin NtAQP1 is a membrane CO2 pore with physiological functions [J]. *Nature*, **425**: 734—737

Walker DW, Miller JC, 1986. Intraspecific variability for drought resistance in Cowpea [J]. *Scientia Horticulturae*, **29**: 87—100

Wang DC, Wu J, Yang YH *et al.*, 2013. Intraspecific variation of leaf epidermal cuticle waxes under scanning electronic microscope: *Stipa purpurea* and *Oxytropis microphylla* from the Qinghai-Tibet Plateau [J]. *Plant Diversity and Resources*, **3**: 348—354

Wang LY, Abbott RJ, Zheng W *et al.*, 2009. History and evolution of alpine plants endemic to the Qinghai-Tibetan Plateau: *Aconitum gymnandrum* (Ranunculaceae) [J]. *Molecular Ecology*, **18**: 709—721

Wang QX, Fan XH, Wang MB, 2014. Recent warming amplification over high elevation regions across the globe [J]. *Climate Dynamics*, **43**: 87—101

Wang ZY, Su G, Luo P *et al.*, 2013. A study of soil-dynamics based on a simulated drought in an alpine meadow on the Tibetan Plateau [J]. *Journal of Mountain Science* **10**: 833—844

Wu GJ, Zhang CL, Xu BQ *et al.*, 2013. Atmospheric dust from a shallow ice core from Tanggula: implications for drought in the central Tibetan Plateau over the past 155 years [J]. *Quaternary Science Reviews*, **59**: 57—66

Wu XL, Shiroto Y, Kishitani S *et al.*, 2009. Enhanced heat and drought tolerance in transgenic rice seedlings overexpressing *OsWRKY11* under the control of HSP101 promoter [J]. *Plant Cell Reports*, **28**: 21—30

Yamaguchi-Shinozaki K, Shinozaki K, 2004. Signal transduction pathways for the activation of ABA-responsive gene expression in drought-stress response [J]. *Plant and Cell Physiology*, **45**: S24—S24

Yamaguchi-Shinozaki K, Shinozaki K, 2006. Transcriptional regulatory networks in cellular responses and tolerance to dehydration and cold stresses [J]. *Annual Review of Plant Biology*, **57**: 781—803

Yang Y, Guo JY, Wang GX *et al.*, 2012. Effects of drought and nitrogen addition on photosynthetic characteristics and resource allocation of *Abies fabri* seedlings in eastern Tibetan Plateau [J]. *New Forests*, **43**: 505—518

Yang Y, Wang GX, Yang LD *et al.*, 2013. Effects of drought and warming on biomass, nutrient allocation, and oxidative stress in *Abies fabri* in eastern Tibetan Plateau [J]. *Journal of Plant Growth Regulation*, **32**: 298—306

Yordanov I, Velikova V, Tsonev T, 1999. Influence of drought, high temperature, and carbamide cytokinin 4-PU-30 on photosynthetic activity of bean plants. 1. Changes in chlorophyll fluorescence quenching [J]. *Photosynthetica*, **37**: 447—457

Yordanov I, Velikova V, Tsonev T, 2000. Plant responses to drought, acclimation, and stress tolerance [J]. *Photosynthetica*, **38**: 171—186

Yue PP, Lu XF, Ye RR *et al.*, 2011. Distribution of *Stipa purpurea* steppe in the Northeastern Qinghai-Xizang Plateau (China) [J]. *Russian Journal of Ecology*, **42**: 50—56

Zhou QY, Tian AG, Zou HF *et al.*, 2008. Soybean WRKY-type transcription factor genes, *GmWRKY13*, *GmWRKY21*, and *GmWRKY54*, confer differential tolerance to abiotic stresses in transgenic Arabidopsis plants [J]. *Plant Biotechnology Journal*, **6**: 486—503

Zhou XM, Wang ZB, Du Q, 1987. *Vegetation of Qinghai* [M]. Xining: Qinghai-People Press

Zhu XH, Bothe O, Fraedrich K, 2011. Summer atmospheric bridging between Europe and East Asia: Influences on drought and wetness on the Tibetan Plateau [J]. *Quaternary International*, **236**: 151—157

Zhuang Q, He J, Lu Y *et al.*, 2010. Carbon dynamics of terrestrial ecosystems on the Tibetan Plateau during the 20th century: an analysis with a process-based biogeochemical model [J]. *Global Ecology and Biogeography*, **19**: 649—662